

## SURFACE TREATMENT STRATEGIES FOR MICROFLUIDIC DEVICES TOWARDS LONGITUDINAL PC12 NEURONAL CELL STUDIES

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### ABSTRACT

We demonstrate polypeptide surface treatments strategies for the promotion of PC12 cell adhesion, proliferation, and differentiation on several MEMS substrates likely to be incorporated into a microfluidic neural interface platform. In particular, a method to successfully clean and reuse microfluidic devices without damaging microfabricated structures following cell culture experiments is described.

**KEY WORDS:** Surface Treatment, Cell Adhesion, PC12, Biocompatibility

### INTRODUCTION

We presented microfluidic MEMS devices that provide focal chemical gradients to targeted cells and localized regions of whole tissue slices [1]. The fabricated cell interface platforms consist of Parylene C microchannels, integrated thermal flow sensors, and SU-8 fluidic interconnects. This microdevice allows study of neuronal cells and tissue subjected to stimulation, differentiation, and recording. Longitudinal cell studies on reusable microfluidic devices require biocompatible surfaces that are durable to conditions of cleaning, culturing, and assessment (pH, temperature cycling, and soaking). For microfluidic flow studies where shear stress may be applied to cells, the surface treatments to promote cell adhesion are necessary, especially when working with non-adherent cell lines [2].

The pheochromocytoma (PC12) cell line is a non-adherent cell type which does not attach well to most materials. It is the most widely used model of neuronal cell function and its differentiation is induced by nerve growth factor (NGF). Its adhesion can be promoted using non-animal derived surface treatments including polyethylenimine (PEI) and poly-D-lysine (PDL) that contain no NGF. Animal-derived treatments (i.e. Matrigel) containing NGF are avoided as they may confound NGF-induced PC12 differentiation studies. Thus, surface treatment strategies using PEI and PDL were investigated on coupons of common MEMS materials and microfluidic devices. PC12 cells were incubated from 1 to 4 days to enable longitudinal studies of focal chemical gradients.

### METHODS AND RESULTS

Test coupons consisted of cell culture substrata (glass and polystyrene (PS)) and MEMS materials (Si, Parylene C on Si (Pa), Parylene on SiO<sub>2</sub> (PaO), Sylgard 184, and medical-grade silicone (MDX4-4210)). For each coupon, four regions on the surface were pre-labeled for repeated measurements. Coupon surfaces and microfluidic devices were cleaned in DI water and sonicated for 5 min.s. Coupons/devices were baked dry (37°C, 30 min.s) and stored at room temperature. Contact angle measurements were performed at each stage of the surface treatment study.

Coupons were separated into 6-well plates and incubated (37°C, 1 hr) in either PEI (100 µM/mL) or PDL (100 µg/mL) [3]. All coupons were triple rinsed with PBS, baked dry (37°C, 30 min.s), and stored at room temperature. Varying storage temperatures (4, 25, 37 °C) did not significantly change contact angles over a 3 day period (data not shown). PEI treatment resulted in a reduced contact angle (more hydrophilic) compared to those of PDL- and pre-treated for PS, Pa, PaO, and Si. Sequential treatments of PEI or PDL were moderately effective (Fig.1 – only PS data shown). No significant contact angle changes were observed in the silicones or glass for either treatment.

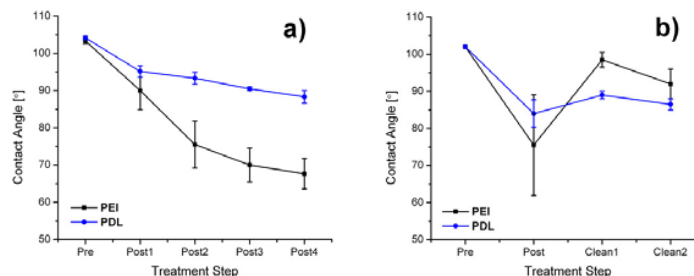


Figure 1: Contact angle data showing (a) stabilization of surface coating following sequential coating treatments and (b) surface coating removal on PS treated surfaces following sequential cleaning treatments.

PC12 cells were thawed from frozen stock and cultured in RPMI culture media supplemented with 7.5% donor horse serum, 7.5% fetal bovine serum, and 0.5% streptomycin/penicillin. Cells were incubated (37°C, 5% CO<sub>2</sub>) in 75 cm<sup>3</sup> culture flasks. PC12 cells (100,000 cells/mL) were then incubated on corresponding polypeptide-coated coupons with or without NGF. NGF (50ng/mL) was added upon plating and every other day for the duration of the experiment. Cell viability was assessed from Day 1-4 using either trypan blue (dead cells) or a fluorescent double-staining assay with Calcein AM (live cells) and propidium iodide (dead cells). Cell viability data was obtained under reflected brightfield and fluorescence microscopy and analyzed using NIH ImageJ. An additional vigorous rinsing step to remove loosely attached cells, which emulates microfluidic agitation generated by focal delivery in the device, dramatically decreased cell density (data not shown). NGF-treated cell cultures were more viable compared to non-NGF-treated cells in all materials tested (Fig.2 – only PaO data shown). Cell densities on both silicones were substantially higher compared to the other materials. Low concentrations of NGF did not induce differentiation and high concentrations (>100 ng/mL) induced differentiation within 22 hrs (data not shown). Localized differentiation by focal delivery of NGF using the microdevice is currently being investigated.

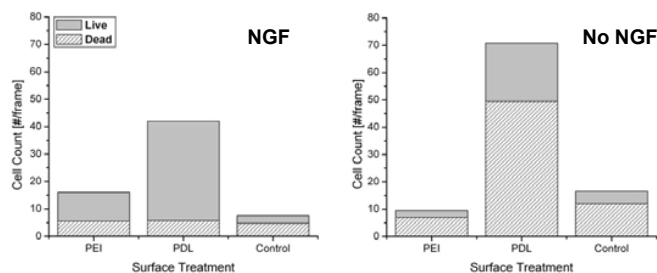


Figure 2: Count of viable PC12 cells labeled with Calcein AM and propidium iodide (PDL-coated, PEI-coated, and uncoated PaO surfaces) (a) with and (b) without NGF treatment.

To clean previously-used coupons/devices covered with PC12 cells and cell debris, coupons/microdevices were treated with trypsin EDTA and incubated for 20 min.s. Coupons/devices were then washed twice with PBS and sonicated in DI water for 5 min.s at a time and repeated sonication as needed (Fig. 3). Device structures survived repeated sonication up to 1 hr total duration before noticeable damage. Passive soaking in DI water or 1M HCl was ineffective in removal of cells and debris compared to active mechanical agitation. Contact angles were restored to native values in all materials that were rendered more hydrophilic by the treatment (Fig 1B – only PS data shown).



Figure 3: PC12 cells grown on PEI-coated glass coupon (a) and untreated microfluidic devices (sensor contact pads shown) (c). Same surfaces shown after cleaning (b,d). A PC12 soma is ~10 μm in diameter.

## CONCLUSIONS

PEI treatment created a more hydrophilic surface that promoted greater PC12 cell adhesion compared to PDL treatment. Priming cell cultures with low concentrations of NGF facilitated adhesion and proliferation of PC12 cells without dramatic induction of differentiation. Cells and debris were successfully removed from test coupons and devices without harsh chemicals or inducing mechanical damage. Methods developed here will enable longitudinal studies of neuronal cultures on microfluidic focal stimulation platforms, especially targeted NGF differentiation.

## REFERENCES

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